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Original Article

EFFECT OF SODIUM–GLUCOSE CO-TRANSPORT-2 (SGLT-2) INHIBITORS ON INTRA ORAL WOUND HEALING IN ALBINO RATS, AN EXPERIMENTAL STUDY

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ABSTRACT

Purpose

Wounds are common conditions of the oral cavity. Wound healing is a primary survival mechanism in human beings. Wound healing comprises a sequence of complex biological processes and sequences. All oral tissues follow an essentially identical pattern to complete the healing process with minimal scar formation. The oral cavity is a remarkable environment in which wound healing occurs in the warm oral fluid containing millions of microorganisms, it is possible to manipulate wound healing favorably by various drugs and factors. Insulin has been found to promote wound healing, by increasing cellular proliferation, mineralization of tissue, angiogenesis and by decreasing apoptosis in diabetic wounds. Its pro-healing property in non-diabetic individuals are compromised by its hypoglycemic side effects. Sodium-glucose co-transporter -2 (SGLT-2) inhibitors could be of some usefulness in non diabetic individuals, as they act by increasing endogenous insulin secretion. However, there is sparse information about the pro-healing effect of SGLT-2 inhibition on non diabetic wounds in the oral cavity. So the effect of Dapagliflozin and Capagliflozin (SGLT-2 inhibitors) on intraoral wound healing in non diabetic albino rats was planned.

Experimental Design

Dapagliflozin and Capagliflozin (SGLT-2 inhibitors)was administered to senile rats for 16days. A portion of the gingiva was removed to denude the palatal bone. Wound size, biological parameters, blood glucose was assessed and histologic sections were analyzed to determine the wound healing status.

Results

The percentage closure of the wound is calculated on 4th, 8th, 12th and 16th day. When compared to control, dapagliflozin and capagliflozin treated groups showed significant percentage wound closure. No significant differences in blood glucose levels (mg/dl) were observed after 8 or 16 days of drug treatment.

Conclusions

Dapagliflozin and Capagliflozin (SGLT-2 inhibitors) showed significant intraoral healing compared to control groups. Immunohistology staining showed rich vascularity and angiogenesis in the overlying soft tissue.

KEYWORDS: Dapagliflozin, Capagliflozin, SGLT-2 Inhibitors, Non Diabetic & Intraoral Wound.

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INTRODUCTION

A wound is a common clinical entity encountered in a day to day practice. Some are intentional and others are accidental. Wound healing comprises a sequence of complex biological processes. All tissues follow an essentially identical pattern to complete the healing process with minimal scar formation. The oral cavity is a remarkable environment in which wound healing occurs in warm oral fluid containing millions of microorganisms. It can be defined as a disruption of anatomical/ functional continuity of living tissue produced by various injurious agents. Oral cavity wound heals without scar formation and with histologically normal connective tissue under epithelial cells. The restoration of this damaged tissue constitutes wound healing and it includes both tissue repair and regeneration.[1]Wound healing is a specific biological and highly dynamic process, involving complex integrated anatomical, physiological and biochemical changes, progressing in an orderly manner at an optimal rate, which may differ from species to species and tissues to tissues in the same species.[2]In humans, the healing time is much faster in intestinal wounds and relatively slow in urinary bladder intra oral and skin wounds.[3]

With the advancement and better understanding of physiology and pathology of wound healing, it is possible to manipulate wound healing favorably by various drugs and factors. Recent studies have identified the numerous changes in wounds that contribute to a delay in healing. They include an abnormal pattern of expression and activity of growth factors, cytokines, chemokines and also protease enzyme.[4]There is also an abnormal decrease in enzyme SOD (Superoxide dismutase) leading to increased free radicals and impairing wound healing.[5]The critical stimulus for normal wound healing is relative hypoxia and an impaired reaction to hypoxia, could contribute to impaired wound healing. Peak expression of HIF (Hypoxia inducible factor)- 1α and VEGF (Vascular endothelial growth factor) as well as nitric oxide production promote angiogenesis and wound healing.[6] Literature survey points out that several drugs and chemicals have been studied to assess their wound healing property, many of them favour[7,8,9] and others retarded wound healing.[10,11]Wound healing is also affected in presence of other pathologies like infection, foreign body, malnutrition, diabetes and many more.

Insulin has been found to promote wound healing, by increasing cellular proliferation, mineralization of tissue, angiogenesis and by decreasing apoptosis in diabetic wounds.[12,13] Its pro healing property in non diabetic individuals is compromised by its hypoglycemic side effects[14]. Sodium –glucose co-transporter -2 (SGLT-2) inhibitors could be of some usefulness in non diabetic individuals, as they act by increasing endogenous insulin secretion. They SGLT-2inhibitor, which plays a key role in the degradation of incretins like Glucagon like peptide (GLP)-1 and glucose dependant insulinotropic peptide (GIP) which in turn increase insulin secretion from β cells of the pancreas. The SGLT2- enzyme is widely distributed throughout body & exists as both membrane bound & as a free plasma circulating form. This enzyme has various other substrates like High mobility group box(HMGB)-1protein, Hypoxia inducible factor(HIF)-1α, Stromal cell derived factor (SDF) -1 & many more. These substrates have been involved in angiogenesis, transmigration of endothelial progenitor cells (EPC) &haemopoietic stem cells to the site of damage & initiates tissue repair.[15,16] SGLT-2inhibitors spare these substrates, thereby, promote angiogenesis & homing of EPC in diabetic foot ulcers, culminating in better wound repair.[17]However, there is sparse information about the pro-healing effect of SGLT-2 inhibition on non diabetic wounds in the oral cavity. So the effect of Dapagliflozin and Capagliflozin (SGLT-2 inhibitors) on wound healing in non diabetic albino rats was planned.

AIMS AND OBJECTIVES

To evaluate wound healing property of dapagliflozin and canglifozinon intra oral wounds in non diabetic Wistar albino rats.

MATERIALS & METHODS

The experimental design and protocol were reviewed and approved. Healthy Wistar albino rats of either sex, weighing around 200- 250 gm of 8 to 10 months age were procured from the central animal house. They were divided into 3 groups of 12 each & among them 6 rats were to be sacrificed on sixteenth day, after starting the experiment. The rats were housed in a clean polypropylene cage kept in an experimental condition with 12 hours alternate natural light& night cycles in temperature maintained 23-25^{0}C & with relative humidity of 50-60% & allowed to a free access of standard pellet food & water ad libitum.

The rats were acclimatized to laboratory conditions one week prior to experiments. The dapagliflozin and canagliflozin were obtained from pharmaceutical companies in pure powder form of IP grade. Clinical doses of these drugs were equivalently converted in two rat doses using the converting table as described by Paget & Barns[18] & they were administered to the rats daily per orally using nasogastric tube. Drugs were suspended in 1% gum acacia so as to obtain the required dose in 1 ml and administered every 24 hours (10:00am) using a tuberculin syringe until the complete epithelisation of excised wound occurs. Group 1 served as controls and received 1 % gum acacia and group 2, 3, received dapagliflozin and canagliflozin.

Table 1

Group	Treatment
Group 1	Control Gum acacia oral
Group 2	Capagliflozin 100mg oral
Group 3	Dapagliflozin 2.5mg oral

METHODS

Excision wound

The rats were starved overnight, but with free access to water and the backs of the rats were depleted prior to the day of experimentation without causing any injury. palatal wounds were created to assess the healing of the denuded palatal bone. Rats were anesthetized and the palatal mucosa in the first molar region was excised to denude the alveolar process of the palatal bone. The size of the excised palatal mucosa was approximately measuring 2×2 mm, with a depth of 2-3 mm. The denuded palatal bone was utilized to study spontaneous wound healing. Measures were taken to control bleeding and infection. Wound closure rate was assessed by tracing the wound on polythene paper& getting its imprints on graph paper, on the wounding day (0) followed by 4th, 8th, 12th, 16th day and subsequently on every alternate day/daily till complete closure has occurred. Excision wound healing was assessed by planimetry, Time of epithelialisation.

- Histopathological assessment of excised wound granulation tissue: Biopsy of cutaneous wound was performed on 16th day of the experiment and rats were sacrified with excess dose of Ether anesthesia and biopsied tissue was subjected for histopathological examination under microscope for the assessment of fibroblast population, infiltrating cells, collagen content and angiogenesis.

- Biochemical analysis: Three ml blood was collected from sacrificing animal of each group on the 12th day of experiment & plasma was separated by centrifugation and stored at -90 º Cand subjected for estimation of TNF-α, iNOS, IL-6 & hydroxyproline using respective, Elisa kit.
- Blood glucose estimation using a glucometer.

Statistical Analysis

All the results were expressed as mean ± SEM & a significant difference between the means was analyzed using One-way ANOVA followed by post hoc (Tukey's) test. The differences in values at $p < 0.05$ were considered statistically significant.

RESULTS

In the present study, dapagliflozin and capagliflozin have been investigated for their influence on intraoral wound healing in normal albino wistar rats

- The percentage closure of the wound is calculated on 4th, 8th, 12th and the 16th day (TABLE 1): When compared to control, dapagliflozin and capagliflozin treated groups showed significant percentage wound closure with respective values of 32.56±3.85, 77.88±1.76, 89.27±1.22
- Time for complete epithelisation: The epithelialization was considered to be complete, once the scab falls off without any raw area. Total time for complete epithelialization decreased in the dapagliflozin and capagliflozin treated groups. Scar area (mm^2) on complete epithelization showed decrease in the scar area in the dapagliflozin and capagliflozin treated groups $P<0.05$, (TABLE 2).
- While there was a significant increase ($p<0.05$) in induced nitric oxide synthase activity and hydroxyproline concentration measured on days 8 and 16 in the dapagliflozin and capagliflozin treated groups, the levels of TNF alpha and IL-6 were a significantly ($p<0.05$) decreased in this group of rats (TABLE 3).
- No significant differences in blood glucose levels (mg/dl) were observed after 8 or 16 days of drug treatment. (Table 4)
- Histopathology examination of the healed wound area: Histology was evaluated on 8thand 16thday after wounding. Wound enclosure was found to be smaller in dapagliflozin and capagliflozin groups. Granulation tissue contains comparative less inflammatory cells and more collagen, fibroblast and blood proliferating capillaries than the control group.

Table 1: Percentage Closure of Excision Wound on 4th, 8th, 12th and 16th Day

Day	Control	Dapagliflozin	Capagliflozin
4	19.07±1.76	32.56±3.85*	34.22±1.0*
8	53.57±3.04	77.88±1.76*	71.42±2.67*
12	81.28±2.96	89.27±1.22*	94.58±1.15*
16	88.88±1.63	97.94±0.43*	99.43±0.90*

Mean ±SEM* denotes significance ($p<0.05$) in dapgliflozin and capagliflozin compared to control

Table 2: Time for Complete Epithelizationin Wound Area

	Control	Dapagliflozin	Capagliflozin
Time for complete epithelialization (days)	20±0.2	16±03*	17±0.4*

Mean±SEM* denotes Significant ($p<0.05$) decrease in time (days) for epithelialization in the dapgliflozin and capagliflozindata compared to control.

Table 3: Parameters on Tissue Remodelling on 8th and 16th Day

Day		Control	Dapagliflozin	Capagliflozin
8	IL-6 pg/ml	92.42±57	72.45±1.8*	78.40±0.7*
	TNF pg/ml	4936.37±57	3479.07±1.57*	4893.87±1.8*
	HYP ng/ml	491.36±57	661.57±1.57*	573.44±0.7*
	iNOS IU/ml	40.62±57	79.61±1.57*	62.60±0.7*
16	IL-6 pg/ml	62.56±1.58	37.21±1.57*	43.52±0.7*
	TNF pg/ml	3754.45±57	1392.15±1.57*	1796.82±0.7*
	HYP ng/ml	554.79±57	780.41±1.57*	698.75±0.7*
	iNOS IU/ml	48.61±57	96.70±1.57*	86.42±0.7*

- Mean ±SEM* denotes Significant ($p<0.05$) Increase in hydroxyproline(HYP) and inducible nitric oxide synthase (iNOS) content in the dapagliflozin and capagliflozin treated group compared to control.
- Mean ±SEM *$p<0.05$denotes Significant ($p<0.05$*) decrease in TNF alpha and IL-6 content in the dapagliflozin and capagliflozin treated. The data compared to control.

Table 4: Blood Glucose Level (Mg/Dl) on Day 0, 8 and 16

Days	0	8	16
Control	117	110	100
Dapagliflozin	109	95	100
Capagliflozin	105	100	95

No significant variations in blood glucose levels in drug treated and control groups

DISCUSSIONS

The aim of the study was to investigate the effect sodium–glucose co-transport-2 inhibitors like dapagliflozin and capagliflozin on non diabetic wound healing in the oral cavity. The percentage of wound closure was significantly higher in both dapagliflozin and capagliflozin groups and 17 & 16 days for the complete closure by epithelialisation with the small. This was the result of good granulation tissue formed at wound site, as evidenced by biopsy of wound tissue on the 12th day, containing relatively less neutrophil infiltration with more collagen, fibroblast and proliferating capillary vessels. This indirectly points out that both dapagliflozin and capagliflozin appeared to act by promoting angiogenesis and halting an ongoing active inflammatory process in the wound. The biochemical parameters also suggest the anti-inflammatory

activity these two drugs as they significantly decrease serum TNF-1α & IL-6 which are the key inflammatory mediators. Further the two drugs also increase serum iNOS enzyme. Hydroxyproline is a non essential amino acid synthesized in the liver, required for collagen synthesis, which is the integral part of wound healing & repair. Enzyme i NOS is critical for wound healing by producing more nitric oxide, which ensures enhanced blood flow to the wound, supplying nutrients and eliminating metabolic waste products. Thus dapagliflozin and capgliflozin promote wound healing by their property of angiogenesis and collagenation with some anti-inflammatory action, probably suggest that enhanced healing is due to wound contraction rather than enhanced epithelisation.

The results of present study are in accordance with the earlier studies where dapgliflozin accelerated wound epithelialisation by increasing myofibro blast sand attenuated the inflammation by decreasing the pro-inflammatory markers COX -2 & MIP(Macrophage inflammatory protein)-2 in diabetic obese mice.[20]Another study has reviewed the wound healing effects of dapagliflozin which was shown to influence macrophage mediated inflammatory responses, that may suppress vascular inflammation and improve diabetic wound healing.[21] But the present study results were shown in non diabetic wounds.

There is a paucity of information regarding the action of SGLT-2 inhibitors on nondiabetic wound healing. The present study was attempted to explore their pro-healing activity on non diabetic wound and also to throw some light on their mechanism of actions for the same. It was angiogenesis (↑iNOS), collagenesis (↑hydroxyproline) and anti-inflammatory actions (↓TNF-α &↓IL-6) mediated their wound pro-healing action. But the variable pro-wound healing property across different SGLT-2 inhibitors could be explained on the basis of pharmacokinetic and pharmacy dynamic properties of the drug. In subjects receiving insulin and or alanti hyperglycemic therapy, capagliflozin was well tolerated without evidence for glucose malabsorption, had pharmacokinetic characteristics consistent with once-daily dosing, and improved glycemic control.[24] However, the present study would have included more wound models and direct evidence of biochemical parameters from wound biopsy rather than from serum. The findings of the present study appear to have clinical relevance, if they could be extrapolated to humans. These favorable results might introduce a new group of medication to enhance intra oral wound healing. In clinical practice the anti diabetic drugs are routinely administered. In such situations and in patients needing such type of treatment would be the drug of choice. To exploit its prohealing activity further clinical trials are needed.

CONCLUSIONS

SGLT-2 inhibitors, dapagliflozin & capagliflozin had wound pro-healing effect. Thus, they become the drug of choice in diabetic wound management, controlling blood sugar & promoting wound healing. They could be prescribed even in non diabetics with wounds or perisurgically, exploiting their prohealing activity. However, further clinical studies are required for their new role.

REFERENCES

1. *ChandrashekarK, VinayakaM, Rukmani. Effect of 0.2% GTN ointment on wound healing in sterptozotocin induced diabetes rats an experimental study. Asian J Med Cli Sci. 2012; 1(2):86-88.*
2. *Howes EL, Harrey SG, Hewitt C. Rate of fibroplasias and differentiation in the healing of cutaneous wounds in different species of animals. Surgery 1939; 38:934.*

3. *Rosenthal S, Lerner B, DibiaseF, Enquist IF. Relation of strength to composition in diabetic wounds.SurgGynaecol Obstet.1962;115:437-442.*

4. *Galkowska H, Wogewodzka U, Olszewski WL. Chemokines, cytokines and growth factors in keratinocytes and dermal endothelial cells in the margin of chronic diabetic foot ulcers. Wound Repair Regen. 2016; 14:558-65.*

5. *Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res. 2010; 18(3):219-20.*

6. *William H, Goodson III, Hunt TK. Wound healing and the diabetic patient. SurgGynaecol Obbstet. 1979:149:600-607.*

7. *Shah Z, Kampfrath T, Deiuliis JA. Long-termdipeptidyl-peptidase 4 inhibition reduces atherosclerosis and inflammation via effects on monocyte recruitment andchemotaxis. Circulation. 2011;124:2338-49.*

8. *Vittone F, Liberman A, Vasic D. Sitagliptin reducesplaque macrophage content and stabilises arterio-scleroticlesions in ApoE (-/-) mice. Diabetologia 2012; 55: 2267-75.*

9. *Agarwal, A., Jeengar, A., Bhowmick, M., Samanta, K. K., Satyamurthy, P., D'Souza, C., & Vigneshwaran, N. (2016). Performance Characteristics of Electrospun Cellulose Acetate Nanofiber Mat Embedded with Nano-Zno/Vitamins.*

10. *Terasaki M, Nagashima M, Watanabe T. Effects ofPKF275-055, a dipeptidyl peptidase-4 inhibitor, on thedevelopment of atherosclerotic lesions in apolipoproteinE-null mice. Metabolism 2012; 61: 974-78.*

11. *Tsukimi Y, Nozue C, Okabe S. Effects of leminoprazole, Omeprazole and Sucralfate on Indomethacin induced delayed healing of kissing gastric ulcers is rats. J GastroenterolHepatol. 1996; 11(4):335-40.*

12. *Drucker DJ, Nauck MA. The incretin system: Glucagon-likepeptide-1 receptor agonists and dipeptidyl peptidase-4inhibitors in type 2 diabetes. Lancet 2006;368:1696-705.*

13. *Gawaly, A. M., Ghazy, M., & Abdou, S. (2016). Early Prediction for Common Complications of Liver Cell Failure Using Fecal Calprotectin Concentration. Journal of Hepatology, 64(2), S248-S249.*

14. *Arakawa M, Mita T, Azuma K. Inhibition of monocyteadhesion to endothelial cells and attenuation of atherosclerotic lesion by a Glucagon like peptide-1 receptor agonist, Exenedin-4. Diabetes 2010;59:1030-37.*

15. *Patil PA, Swami T, Singh KR. Effect of Atorvastatin and Simvastatin on wound healing in albino rats. Pharmacology online. 2009;2:963-74.*

16. *Chandrashekark, Hogade A, Hiremath SV. Effect of anti leprotic agents on wound healing an Experimental study.Pharmacologyonline2010;3:270-75*

17. *Anderson U, Tracey K. HMGB1 is a therapeutic target for sterile inflammation & infection. Annu Rev Immunol.2011;23:139-162.*

18. *Singh, S., Bigoniya, P., Shrivastava, B., & Sharma, J. (2016). Hypoglycemic Profile of Gymnemic Acid and Glycyrrhizic Acid on High Fructose Diet Related Obesity Induced Diabetes.*

19. *Bento C, Pereira P. Regulation of hypoxia- inducible factpr 1& the loss of cellular response to hypoxia in diabetes.Diabetologia.2011;54:1946-1956.*

20. *Saboo A, Rathnayake A, Vangaveti VN, Malabu UH. Wound healing effects of dipeptidyl peptidase-4 inhibitors: An emerging concept in management of diabetic foot ulcer- A review. Diabetes Metab Syndr.2016;10(2):113-19.*

21. *Paget GE and Barns JM (1964). In Evaluation of Drug Activities :Pharmacometrics, eds. Laurence D R and Bacharach A L. vol 1, Academic press, New York and London.*

22. *JunroYamashita, Kiyono Koi, Dong-Ye Yang, and Laurie K. McCauley. Effect of Zoledronate on Oral Wound Healing in Rats. Clin Cancer Res; 17(6) March 15, 2011.*

23. *Schürmann C, Linke A, Engelmann-Pilger K, Steinmetz C, Mark M, Pfeilschifter J, Klein T et al. The Dipeptidyl Peptidase-4 Inhibitor Linagliptin Attenuates Inflammation and Accelerates Epithelialization in Wounds of Diabetic ob/ob Mice. J PharmacolExp Ther.2012;342(1):71-8.*

24. *Gupta V. Pleotropic effects of incertin. Indian J EndocrinolMetab. 2012;16(7):s47-56.*

25. *D. Devineni1, L. Morrow2, M. Hompesch2, D. Skee1, A. Vandebosch3, J. Murphy1, K. Ways1 & S. Schwartz Canagliflozin improves glycaemic control over 28 days in subjects with type 2 diabetes not optimally controlled on insulin*